

Quantitative aspects of the latex-fixation and Waaler-Rose tests

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The latex-fixation test (Singer and Plotz, 1956) is widely used as a serological criterion for rheumatoid arthritis (Alexander and McCarthy, 1966; Walton, 1968). The test is often carried out in serial dilution, by which a titre as well as an agglutination pattern is obtained. The usual practice is to choose a borderline titre in an empirical way and to call all titres above the borderline value positive and below it negative. Little attention has been paid in the literature to the possible significance of the titre value irrespective of this borderline. Population studies suggest that the presence and titre of rheumatoid factors may be related to the severity of rheumatoid disease (Valkenburg, Ball, Burch, Bennett, and Lawrence, 1966; den Oudsten, Planten, and Posthuma, 1968). Several authors (Jacobson, Kammerer, Wolf, Epstein, and Heller, 1956; Ball and Lawrence, 1963; Hill and Greenbury, 1965; Cats and Hazevoet, 1970) have demonstrated a close correlation between a positive haemagglutination or latex-fixation test and the presence of subcutaneous nodules, radiographic evidence of articular erosions, and stage of disease. There are, however, many examples in the literature of individual cases in which the titre values did not follow the course of the disease.

The agglutination pattern has recently received some attention (Schubart, Cohen, and Calkins, 1964). It has been established that some sera contain thermolabile components which influence the titre and the agglutination pattern in the latex-fixation test. Thus, in serial dilutions of positive rheumatoid sera, agglutination may be absent in the initial tubes, resulting in a pattern which resembles the prozone of certain other antibody-antigen reactions. This phenomenon has been studied in our laboratory (Valkenburg and de Mos, 1958; Klein, 1964). The inhibitor is thermolabile and is probably identical to the first component of complement. Previous heat inactivation of serum abolishes the prozone phenomenon and may enhance agglutination. The presence of the inhibitor may conceal possible agglutinators of the rheumatoid factor type (Klein, Valkenburg, van Zwet, and Lafeber, 1966).

The present study was undertaken to investigate whether titre values and prozone phenomena could provide additional data of interest for the clinical evaluation and biological significance of the latex fixation test. Some results obtained with the Waaler-Rose test are also discussed.

Material and methods

LATEX-FIXATION TEST

This was performed by a modification of the Singer and Plotz technique (Valkenburg 1963a), in which serial dilutions are carried out in glycine buffer with 0.1 M NaCl. After incubation at 56°C., the tubes are left overnight at room temperature before centrifuging. Control tubes must be free of sediment as the usual practice of 'flipping' the tubes to disperse false positive precipitates also abolishes true weak agglutinations. The stock latex suspension (Difco Bacto Latex 0.81 μ) was stabilized by adding bovine serum albumin to the diluting glycine buffer to a final concentration of 0.4 per cent., because it was observed that some batches of Difco Latex suspension had a tendency to agglutinate spontaneously in glycine buffer alone, resulting in positive readings in control tubes. The albumin must be added to the diluting glycine buffer on the same day as the test is performed. A 0.6 per cent. dilution of the stock latex suspension was used instead of the 1 per cent. dilution in the Singer and Plotz procedure. In clinical practice all sera with a titre of at least 1 : 640 and without prozone are considered to be positive.

For the routine latex-fixation test sera were *not* inactivated previously. In some instances sera were inactivated by heating the samples at 56°C. for 30 min. in a water bath before performing the test. Whenever this was done, it is specifically mentioned.

WAALER-ROSE TEST

This was carried out with human O Rh + erythrocytes sensitized with rabbit antibody (Valkenburg, 1963b). In clinical practice all sera with a titre of at least 1 : 32 were considered to be positive.

The sheep cell test was not used in our studies. No comparison with the International Reference Rheumatoid Arthritis serum was made for either the latex test or the Waaler-Rose test.

SERA

Sera from all in-patients and out-patients, regardless of diagnosis, who visited our department during a 3-year period (1958-1959-1960) and who had a latex fixation titre equal or above 1 : 20, were studied.

Every new serum sample, whether or not from a patient seen before, was considered as a separate case, in order to avoid selection by biased choice. However serological examinations are often repeated in order to confirm an unexpected positive or negative answer and this procedure could therefore introduce a new bias. In order to test this possibility, all first, second, etc., readings from patients examined more than once were correlated separately with the number of American Rheumatism Association (ARA) criteria. In all instances the same distribution as in the total sample was found, which justified the procedure outlined above.

A total of 1,661 sera from 1,030 persons was studied, 430 of which showed a prozone effect. The negative group was composed of every tenth patient with a titre < 1 : 20 who visited our department in the same period (294 sera).

The titre obtained was correlated with the number of ARA criteria, the diagnosis of osteoarthritis and non-articular rheumatism, the presence of nodules, radiological changes of rheumatoid arthritis of grade 2 or more (Steinbrocker), and the erythrocyte sedimentation rate (Westergren).

Of the 1,955 sera investigated by the latex-fixation test, only 1,311 were investigated by the Waaler-Rose test as well, because this test was introduced later in our laboratory.

Table I Comparison of male and female patients with latex-fixation titres. Titre \geq and < 640

Latex-fixation test	Male	Female	Total
Titre \geq 640	469	863	1332
Titre < 640	182	441	623
Total	651	1304	1955

$\chi^2_1 = 6.8724$; $0.005 < P < 0.01$

Results

SEX AND AGE DISTRIBUTION IN THE LATEX-FIXATION TEST

Of the total number of 1,955 latex-fixation tests, 651 were from male and 1,304 from female patients (Table I). Male and female patients were distributed in the same proportions in each of the four age groups (Table II). 469 of the males (72.1 per cent.) had a titre of 1 : 640 or more, whereas 863 of the females (66.2 per cent.) came within this range. This difference (which is statistically significant with $\chi^2_{(1)} = 6.8724$; $0.005 < P < 0.01$) is due to the higher percentage of male patients with a titre equal to and over 1 : 5120 in comparison with the percentage of female patients with this titre. The percentages of male and female patients with a titre < 1 : 20 were about the same (male 14.7 per cent.; female 15.2 per cent.).

The percentage of patients (male and female) with lower titres (< 1 : 20 and 1 : 20-320) was higher in the age groups below 25 years and 25 to 44 years old than in the older age groups (Table II).

No difference for the titres 640, 1280, and 2560 was found in the four age groups. The largest proportion of patients with a titre 5120 and \geq 10240 was in the group from age 45 onwards, but for the male patients there was a decrease again in the age group \geq 65 years. There was no difference in the titre distribution for the female patients between the age groups 45 to 64 and 65 to 84 years.

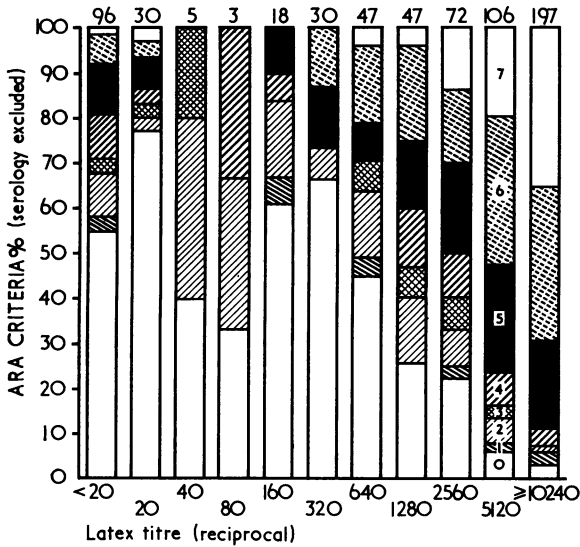
RELATIONSHIP OF LATEX-FIXATION TEST AND WAALER-ROSE TEST TO ARA CRITERIA FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS

Only 18.8 per cent. of male and 23.7 per cent. of female patients with a titre < 1 : 20 (Fig. 1) had five or more ARA criteria. These percentages increased gradually from titres of 160 upwards. Up to 1 : 2560 there were more females than males with \geq five ARA criteria ($\chi^2_{(1)} = 15.17725$; $P < 0.0005$) but from 5120 upwards there were slightly more males with this number.

Table II Distribution (per cent.) of titre values in four age groups, by sex

Age group (yrs)	< 25		25-44		45-64		65-84	
	male per cent.	female per cent.	male per cent.	female per cent.	male per cent.	female per cent.	male per cent.	female per cent.
LFT								
<20	31	36	20	16	11	14	18	11
20-320	31	24	21	29	10	16	10	11
640	19	20	8	10	6	8	10	13
1280	13	8	7	11	6	13	11	13
2560	6	4	11	12	12	13	9	16
5120		6	14	7	18	14	16	14
\geq 10240		2	19	16	37	22	26	22
Total number	16	74	143	315	387	679	105	236

MALES (651)



FEMALES (1304)

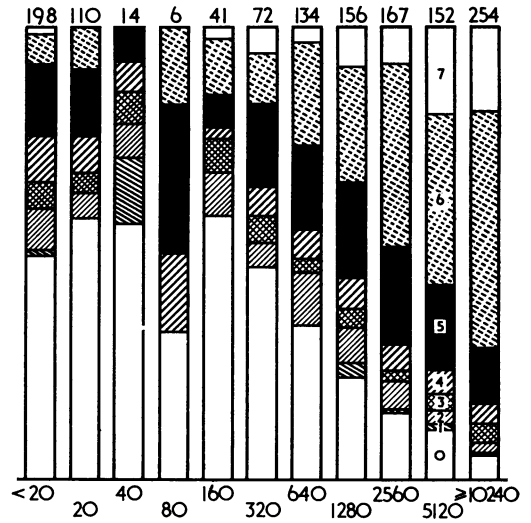


FIG. 1 Relationship of latex-fixation titre to ARA criteria in tests in males and females. Figures 0 to 7 in diagram refer to number of ARA criteria

The incidence of rheumatoid nodules and of radiological erosions increased with titres of 1 : 80 upwards, as is shown in Fig. 2. Rheumatoid nodules and erosions were found in 30 and 80 per cent. respectively of the patients with titres \geq 5120.

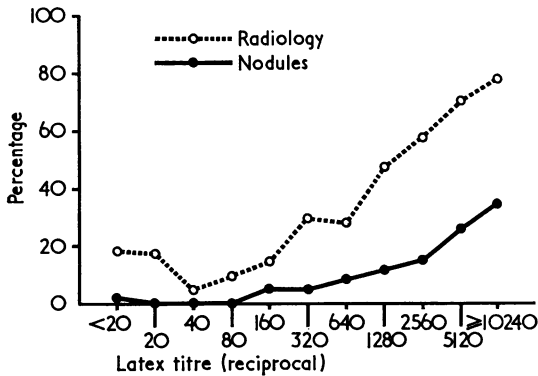


FIG. 2 Relationship of latex-fixation titre to clinical criteria.

In Fig. 3 the erythrocyte sedimentation rate (ESR) of the 899 patients with definite RA was compared with the latex-fixation test as a criterion of the disease. Although the percentage of patients with a titre $>$ 1 : 80 was independent of the ESR, there were progressively more titres $>$ 1 : 5120 with a rising ESR.

In our hands the Waaler-Rose test is less sensitive but more specific than the latex-fixation test. From the 118 patients with a titre of 1 : 16 and 1 : 32, 21 per cent. had rheumatoid nodules and 69 per cent. had x-ray abnormalities (Fig. 4). These figures

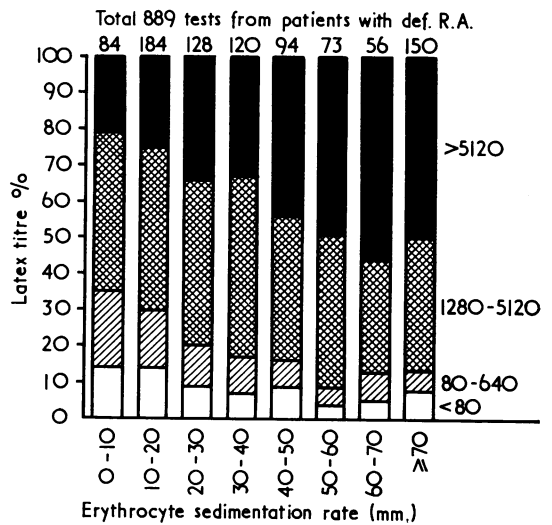


FIG. 3 Relationship of latex-fixation titre to erythrocyte sedimentation rate.

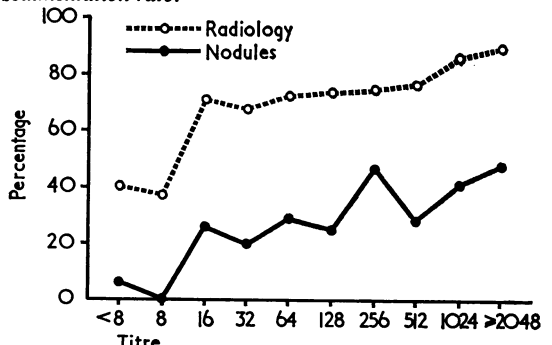


FIG. 4 Relationship of Waaler-Rose titre to clinical criteria.

increased to 44 and 86 per cent. respectively in the 117 patients with a titre of 1 : 1024 or more; 6.5 per cent. of 561 patients with a titre < 1 : 8 had nodules. With regard to these criteria, the Waaler-Rose test therefore presents essentially the same picture as the latex-fixation test. This is also true for the correlation of Waaler-Rose titres with ARA criteria and ESR.

CORRELATION OF AGGLUTINATION PATTERN AND CLINICAL DATA

A prozone or interzone phenomenon was observed in 430 latex-fixation tests. A gradual increase in the percentage of patients with five or more ARA criteria in relation to titres of 1 : 320 or greater was noted in this group as well. This correlation was equally striking for rheumatoid nodules and radiological lesions; 60 per cent. of the 45 patients with titres of \geq 1 : 5120 showed radiological erosions and 24 per cent. had rheumatoid nodules. When patients

Table III *Number of ARA criteria in patients with a latex-fixation titre of 640 and 1280, with and without prozone phenomenon*

No. of ARA criteria	LFT titre 640 and 1280		Total no.
	prozone +	prozone -	
0 + 1	77	45	122
2 + 3	27	30	57
4	13	14	27
5 + 6 + 7	73	105	178
Total no.	190	194	384

$\chi^2_{(3)} = 14.3016; 0.001 < P < 0.005$

with titres of 1 : 640 and 1 : 1280 with and without a prozone phenomenon were compared (Table III), a difference was found between the number of patients with one criterion or none and those with two and three, four and five, or more criteria. The group of patients in whose sera no prozone phenomenon was found, contained significantly more cases of rheumatoid arthritis. Thus the chance ($\chi^2_{(3)} = 14.3016; 0.001 < P < 0.005$) of having rheumatoid arthritis is less for a patient whose serum shows a prozone effect with a titre of 1 : 640 or 1 : 1280 than for another patient with the same latex titre without such a prozone phenomenon.

The significance of the prozone effect also appears from the fact that we found a prozone phenomenon in 36 per cent. of a group of 320 patients with clinically proven osteoarthritis and non-articular rheumatism, compared to 17 per cent. in a group of 775 cases of probable, definite, or classical rheumatoid arthritis. ($\chi^2_{(1)} = 45.2621; P < 0.0005$).

Some experiments were carried out to test the hypothesis put forward by Schubart and others (1964) that the extent of the prozone phenomenon does not depend upon the rheumatoid factor titre. Equal amounts of different inactivated sera with increasing latex titres were added to equal amounts of the same negative serum as a source of inhibitor and measured together in the latex-fixation test. It had been shown previously that prozone phenomena can be produced in this way (Klein, 1964). Some typical results are shown in Table IV, where it is seen that the inhibitor zones decrease with increasing rheumatoid factor concentrations.

FREQUENCY DISTRIBUTIONS

When the titre frequencies of the 1955 tests were plotted against the titre values, a biphasic curve was found (Fig. 5). The first peak is formed by the negative sera (titre below 1 : 20). A minimum is observed at titre values around 1 : 80. The second

Table IV *Interaction of rheumatoid factor and inhibitor in the latex-fixation test*

Sera	Dilution series (reciprocal)									
	20					→10240				
	0.1 ml. positive serum									
						0.1 ml. positive serum + 0.15 ml. negative serum				
A	±	±	—	—	—	—	—	—	—	—
B	2	2	(+)	±	—	—	—	—	—	—
C	2	2	2	2	2	±	±	—	—	—
D	2	2	2	2	2	2	±	—	—	—
E	3	2	2	2	2	2	2	1	—	—
F	2	2	2	2	2	2	2	2	1	±

Evaluation of agglutination in each tube
 { ± trace of agglutination
 (+) incomplete agglutination ring
 1 complete agglutination ring
 2, 3 increasing amounts of agglutination

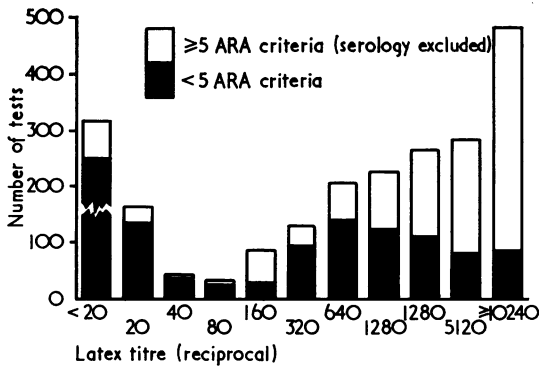


FIG. 5 Distribution of latex-fixation titres. Broken bar indicates random choice of group with titre < 20.

peak may have its maximum around 1 : 10240, but this does not show in the graph, since 10240 was the last dilution in the titration. Furthermore it is apparent that the number and proportion of patients with definite rheumatoid arthritis increases with rising titre.

With the Waaler-Rose agglutination test, the same biphasic curve was found (Fig. 6), but here the number of tests that were negative or showed a titre below 1 : 16 was relatively greater. The second part of this graph, with a peak at a dilution of 1 in 128, seems to form the curve of a normal distribution. This graph also shows that there was a high proportion of patients with five or more ARA criteria and a Waaler-Rose titre < 1 : 8.

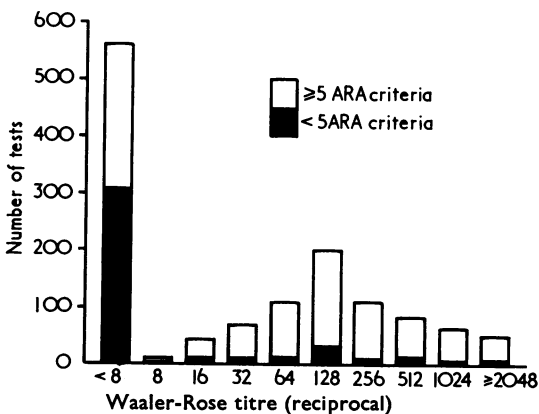


FIG. 6 Distribution of Waaler-Rose titres

When the latex test titres of the patients with degenerative joint disease (osteoarthritis and disc degeneration) and those with non-articular rheumatism (e.g. painful shoulder, tennis elbows) were plotted in the same way, a biphasic curve (Fig. 7) again appeared, but here the maximum of the second phase occurred at a titre of 1 : 640.

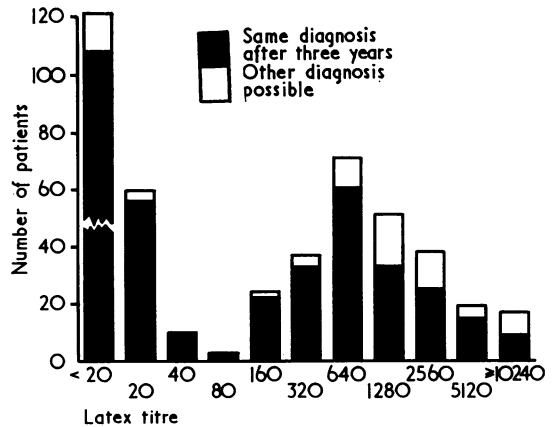


FIG. 7 Distribution of latex-fixation test in osteoarthritis and nonarticular rheumatism.

Broken bar indicates random choice of group with titre < 20.

In a follow-up study 3 years later, the original diagnosis of degenerative joint disease and non-articular rheumatism were reassessed. In the sera of two patients with a latex titre of 1 : 1280 and 1 : 10240 respectively, LE-cells were found. In none of the other patients had definite rheumatoid arthritis or any other connective tissue disease developed. In 76 of the 452 cases, the x-ray pictures of hands, feet, shoulders, and cervical spine showed abnormalities as compatible with the diagnosis of rheumatoid arthritis as with the diagnosis of osteoarthritis and disc degeneration, such as narrowing of the joint space, small cysts with a sclerotic wall, or cervical disc degeneration without evidence of osteophytes. Erosions at the joint surface were never seen however. Thirteen of these 76 patients had swelling of one or two joints during shorter or longer periods, sometimes with morning stiffness. Six of them had enough positive criteria for a diagnosis of 'probable rheumatoid arthritis'.

Excluding all these 76 patients (Fig. 7, white bars), the curve remained biphasic with the maximum at a titre of 1 : 640. In 76 cases some evidence for the diagnosis of RA was present. This group of 76 cases was composed of 25 of 256 cases with a titre of < 1 : 640 and 51 of 196 cases with a titre ≥ 1 : 640. Thus, there are significantly more cases ($\chi^2_{(1)} = 19.8238$; $P < 0.0005$) with titres ≥ 1 : 640 and some radiological abnormalities in which the diagnosis RA can not be excluded, than there are with titres 1 : 640.

The question arose whether the thermolabile inhibitor which can be found in most sera is responsible for the biphasic pattern in the latex-fixation test. To test a possible effect of this thermolabile inhibitor, the following experiment was carried out:

Blood was taken from 537 consecutively chosen patients who visited our department between February and May, 1964. A latex-fixation test was carried out

with native sera and after inactivation by incubation of the sera during 30 min. at 56°C. Fig. 8 shows that the biphasic shape of the frequency distribution is lost after heat treatment.

Discussion

SEX AND AGE IN RELATION TO LATEX TITRE

Although RA is considered to be predominantly a disease of females and the ratio of males to females in our sample was 1 : 2, the proportion of males with high latex titres ($\geq 1 : 5120$) was significantly higher than that of the females. It seems as if the males, once they have contracted the disease, react more violently than the females, which is contrary to the expectation that the latter are more prone to auto-immune phenomena. Ball (1952) and Sievers (1965) also found more male than female patients with a high titre in the haemagglutination test. Using the latex-fixation test, Valkenburg and others (1966) found more females (2 per cent.) than males (1 per cent.) with a titre of 1 : 5120 or more in a population study.

It is possible that the male patients had more severe RA and that their life expectancy was shorter, since the highest percentage of latex titres in males was found in a younger age group (45 to 64 years) than in females. The high proportion of positive latex tests in females > 50 years might alternatively be explained as the consequence of the fact, observed by Short, Bauer, and Reynolds (1957) and by Kellgren (1968), that the incidence of RA in women increases markedly above the age of 50 years.

In the above-mentioned population study, Valkenburg and others (1966) also found that the percentage of males with a latex titre $\geq 1 : 5120$ did not increase in the age group > 45 years. For the

females the maximal incidence of titres ≥ 5120 was reached at the age of 65 years. Waller, Toone, and Vaughan (1964) found a higher incidence of positive rheumatoid serology in the age group over 60 years, but in their group men and women were taken together and the latex test used in their study was the Hyland slide test which is not directly comparable with our test.

LATEX FIXATION TITRE—ARA CRITERIA—ESR

The aetiology of rheumatoid arthritis is still obscure. Certain signs and symptoms have been accepted to define the syndrome, and certain serological tests for the estimation of rheumatoid factor in the serum and the analysis of the synovial fluid are at present the only laboratory procedures which can be helpful in making a diagnosis.

It must be considered whether rheumatoid factor occurs in the serum of patients with RA as an independent phenomenon or whether it is related to the other criteria used for the characterization of rheumatoid arthritis. From our study it appears that from a latex titre of 1 : 160 upwards the number of male and female patients with five or more ARA criteria gradually increases. Thus there is a correlation between the titre values and the number of ARA criteria. Such a positive correlation has also been found between the latex titre and two very specific symptoms, the prevalence of the rheumatoid nodules and radiological abnormalities of the joints. Similar correlations have been found with the Waaler-Rose test. With a sensitive serological test for rheumatoid factor it is therefore possible to estimate at every titre level the chance that other features of RA will be present.

There is general agreement that in patients with rheumatoid arthritis and an elevated ESR the serological tests are more frequently positive than in

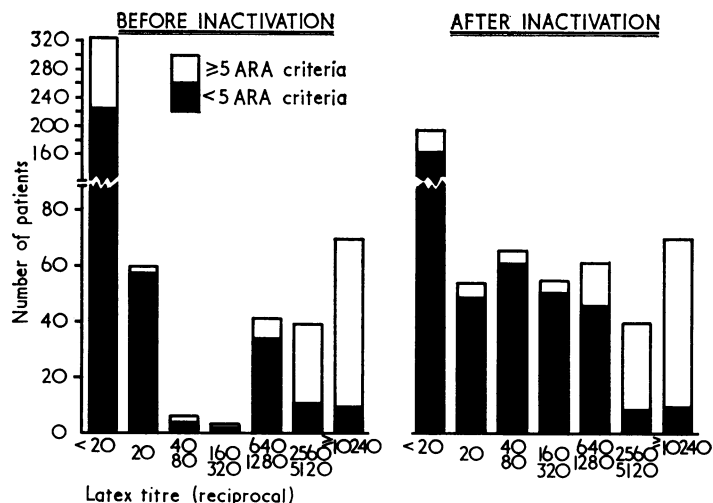


FIG. 8 Distribution of latex-fixation titres before and after inactivation of serum.

patients with a normal or only slightly raised ESR. Our data confirm that with a rising ESR relatively higher latex titres are found. It seems reasonable to assume that the ESR as well as the latex titre reflects the disease activity, or the extension of the process, or both.

In the literature little attention has been given to titre values because serological results are usually expressed as positive or negative only (Jacobson and others, 1956; Robecchi and Daneo, 1959; Ball and Lawrence, 1963; Wager, Ripatti, Laine, Julkunen, and Aho, 1961). Cobb (1963) has already suggested that rheumatoid factors should be thought of in quantitative terms rather than as substances that are either present or absent. Our material and the additional data in the literature indicate that the titre value is dependent on factors such as age, sex, and the number of signs and symptoms present. These latter findings make it understandable that in population studies (den Oudsten and others, 1968) about 30 per cent. of the patients with definite RA have a positive titre, while in patients in a department of rheumatology a positive test has been found in about 75 per cent. The reason for this may be that the patients who come to a specialized clinic are often more seriously ill than those examined in population surveys. There are no indications that we are dealing with different diseases, sero-negative and sero-positive RA.

AGGLUTINATION PATTERN

It has been observed (Olsen and Rantz, 1958; Valkenburg and de Mos, 1958) that in the latex-fixation test agglutination may fail to occur in the presence of high concentrations of serum, sometimes resulting in a pattern resembling the prozone of some antigen-antibody reactions. This prozone phenomenon is due to the presence of a thermolabile inhibitor, which was identified as the first component of the serum complement system (C'_1). This thermolabile component is able to inhibit the reaction between the γ globulin coated latex particles and small concentrations of rheumatoid factor (Schubart, 1959; Klein, 1964; Klein and others, 1966). The observation of the prozone phenomenon may have some significance for the clinician, since, the rheumatoid factor titres being equal, significantly fewer ARA criteria are found in patients in the prozone group, as appears from Table III. In this connection it is interesting to note the observation of Laurell and Grubb (1958) that C'_1 levels are not significantly changed in rheumatoid arthritis. Ellis and Felix-Davies (1959), however, found an increase in C'_1 in twenty RA patients. No correlation of either inhibitor or severity of the disease with total complement levels was found in a longitudinal study by Schubart, Ewald, Schroeder, Rothschild, Bhatavadekar, and Pullen (1965).

Schubart and others (1964) claimed that prozone phenomena were more often found in patients with temporary remissions. These patients also had lower latex titres, but these authors suppose that the extent of the prozone phenomenon does not depend upon the concentration of the rheumatoid factor. The concentration of the rheumatoid factor and of the inhibitor could therefore be estimated quantitatively in the same way. We were able to show, however, that the titre of the rheumatoid factor, as revealed after inactivation, does in fact influence the extent of the prozone phenomenon. This means that the two are interdependent and that with lower titres more prozone phenomena can be expected to occur. The interaction between rheumatoid factors and inhibitor is a consequence of their binding to neighbouring sites on the Fc fragment of the IgG molecule (Heimer, Levin, and Kahn, 1963; Klein, 1964).

Inactivation of the inhibitor reveals many sub-clinical titres, especially in the group with \geq five ARA criteria, whose sera nearly always give a titre of at least 1 : 20. After inactivation many normal and otherwise negative human sera also appear to contain small amounts of rheumatoid factor.

FREQUENCY DISTRIBUTIONS

The biphasic character of the frequency distribution curve of the latex-fixation test suggests the presence of two different populations of rheumatoid factors, one in low concentrations occurring mainly in the non-rheumatoid group, and one in high concentrations representing the rheumatoid arthritis group. Our experiments with heat-treated sera make such a supposition improbable. The dip in the frequency distribution curve at titre values of 1 : 40 to 1 : 640 is caused by the presence of the thermolabile inhibitor, and the resulting curve may be part of one distribution function only, in which the RA patients are found at the extreme right. This distribution reflects the gradual increase of ARA criteria with increasing titre. The biphasic frequency distribution obtained by Mongan, Cass, Jacox, and Vaughan (1969) with heat-inactivated sera in the latex-fixation test remains unexplained. Our results do not support their contention that sero-negative RA and sero-positive RA form different populations, but rather demonstrate the continuity of the occurrence of rheumatoid factor over the whole range from diseased controls to classical cases of RA. Similar conclusions were reached by Zutshi, Reading, Epstein, Ansell, and Holborow (1969) on the basis of a F II haemagglutination test.

It is possible that a comparable situation exists in the Waaler-Rose test, where the thermostable inhibitors have been demonstrated (Franklin, 1960; Ziff, Brown, Lospalluto, Badin, and McEwen, 1956).

The distribution curve of 452 patients with osteoarthritis, spondylarthritis, disc degeneration, and non-articular rheumatism has its maximum at the titre of 1 : 640. The shape of this curve did not alter when all patients in which there was some evidence for another diagnosis after a follow-up period of 3 years were excluded.

Differences in the test systems make it understandable that the frequency distributions published by Amira and Visconti (1959), Wager and others (1961), and Zavázal and Lavička (1962) show small differences from our curves. In population studies (Ball and Lawrence, 1961) such curves have not been found, probably because in such surveys the number of positive reactions is relatively small.

GENERAL DISCUSSION

The investigations described in this paper have been carried out to provide an answer to the question whether there is any use in performing a quantitative latex-fixation test. The significance of our findings can be discussed from two points of view.

(1) *The significance in relation to the theoretical background of rheumatoid serology*

A parallel exists between the occurrence of ARA criteria and rheumatoid factor without an absolute correlation, for individual cases are found in which six ARA criteria can co-exist with a latex titre of 1:160, etc., (Fig. 1). Rheumatoid factor, therefore, cannot be considered as the direct cause of the pathological phenomena of RA, nor as directly connected to their cause, as is also demonstrated by the existence of so-called seronegative RA. In fact, the low titre part of the frequency distribution curve of the latex factor must extend well into the domain of normal subjects, since small amounts of these factors can be shown to exist in a considerable number of normal healthy persons (Klein and others, 1966). This means that the rheumatoid factor is a more general phenomenon in immunology than has previously been supposed. Thus one can find measurable titres in unrelated diseases like osteoarthritis (Fig. 7), and also in some chronic infectious diseases like subacute bacterial endocarditis (Williams and Kunkel, 1962) and trypanosomiasis (Klein and Mattern, 1965), although in the latter case it is only the Waaler-Rose factor which is increased. In these cases the rheumatoid factors may be considered as antibodies against γ globulin which, because of its reactions with certain antigens, has been altered in such a way as to become antigenic itself.

This view has been confirmed by the experimental production of rheumatoid factors in animals under circumstances of chronic immunization (*e.g.* by Abruzzo and Christian, 1961). The rheumatoid factors should therefore not be considered as the

expression of an abnormal reactivity of the immunological system, but rather as a more or less normal reaction which can be detected with greater clarity in some abnormal situations. The nature of such a situation in RA remains unknown. It may be exogenous, but autoimmunity cannot be excluded, although the rheumatoid factor itself need not implicate an autoimmune disease: it can be a side-product. The parallelism of the rheumatoid factor with clinical phenomena as well as with the ESR suggests that it is a general indicator of activity and extension of the disease, although with somewhat greater specificity. It is this increased specificity that gives rheumatoid serology its limited use.

(2) *The practical advantage in applying the serological tests for RA in a quantitative way*

The number of signs and symptoms of the disease increases with a higher titre and also the prognosis of RA patients with low or negative titres is better than of those with high titres (Cats and Hazevoet, 1970). Thus, the natural history of the disease is less favourable in the presence of high serum titre. In giving medical and social advice to the patient, we take the titre values together with the prozone phenomena into consideration as a prognostic sign in the same way as the presence of serious, erosive lesions of the joints.

It therefore seems desirable at present to maintain a quantitative serological test for rheumatoid factor with the possible prozone, firstly to care for our patients in the best possible way and secondly to help us to understand the role of the immunological phenomena in this disease.

Summary

(1) During a 3-year period, 1,661 sera with a latex-fixation titre equal to or above 1 : 20 from 1,030 persons and 294 sera chosen at random with a titre below 1 : 20 were sampled.

(2) The relationship between the titre value and a number of parameters (age, sex, number of ARA criteria, nodules, radiological abnormalities, and ESR) was studied.

(3) There were relatively more males than females with high latex titres. The highest percentage of males with titres \geq 1 : 5120 were found in the age group 45 to 64 years, and after 65 years there was a decrease. No difference was found in the titre distribution of the female patients between the age groups 45 to 64 and 65 to 84 years.

(4) The percentage of male and female patients with five or more ARA criteria, nodules, and radiological abnormalities steadily increased with titre values rising from 1 : 160. With a rising ESR, the percentage of patients with five or more ARA criteria

and a latex titre > 1 : 5120 increased.

(5) The patients in whose sera a prozone phenomenon had been found had fewer ARA criteria than those with the same end-titre but without a prozone phenomenon.

(6) The titre frequencies of all sera showed a biphasic curve. The dip in the frequency distribution curve is caused by the presence of a thermolabile inhibitor and disappears after inactivation of this inhibitor, revealing a graded continuum from diseased controls to cases of classical rheumatoid arthritis.

(7) The frequency of titres in sera from patients with osteoarthritis and nonarticular rheumatism also showed a biphasic distribution. A follow-up study revealed that, in these patients, more radiological abnormalities were present when they had higher latex titres.

(8) There is evidence that these conclusions are also valid for the Waaler-Rose test.

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